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Autoimmunity and COPD: clinical implications

Gaetano Caramori, MD, Paolo Ruggeri, MD, Antonino Di Stefano, PhD, Sharon Mumby, PhD, Giuseppe Girbino, MD, Ian M. Adcock, PhD, Paul Kirkham, PhD

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Gaetano Caramori, MD, Paolo Ruggeri, MD, [§]Antonino Di Stefano, PhD, °Sharon Mumby, PhD, Giuseppe Girbino, MD, °Ian M Adcock, PhD, *Paul Kirkham, PhD

Unità Operativa Complessa di Pneumologia, Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali (BIOMORF), University of Messina, Italy, [§]Divisione di Pneumologia e Laboratorio di Citoimmunopatologia dell'Apparato Cardio Respiratorio, Istituti Clinici Scientifici Maugeri, SpA, Società Benefit, IRCCS, Veruno (NO), Italy, °Airways Disease Section, National Heart and Lung Institute, Imperial College London, UK, and *Department of Biomedical Sciences, Faculty of Sciences and Engineering, University of Wolverhampton, UK

Corresponding author information: Prof. Gaetano Caramori, MD, PhD, Unità Operativa Complessa di Pneumologia, Dipartimento di Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali (BIOMORF), Piazza Pugliatti 1, 98122, University of Messina, Italy, E-mail: gcaramori@unime.it

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Abbreviation list: BAFF=B-cell activating factor; BALT=bronchus-associated lymphoid tissue**;** COPD=chronic obstructive pulmonary disease; Ig=immunoglobulin; IL=interleukin; LF=lymphoid follicles; mAbs=monoclonal antibodies; Th=T-helper;

Abstract

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. Long term cigarette smoking is the cause of more than 90% of COPD in Westernized countries. However, only a fraction of chronic heavy smokers develop symptomatic COPD by the age of 80 years. COPD is characterized by an abnormal immune response in the lower airways and its progression is associated with infiltration of the lung by innate and adaptive inflammatory immune cells that form lymphoid follicles. There is growing evidence that both cellular- and antibody-mediated autoimmunity has a fundamental role in the pathogenesis of stable COPD. In particular, carbonyl-modified proteins may help to drive autoimmunity in COPD and to cause the characteristic small airways abnormalities and even contribute to the pathogenesis of pulmonary emphysema. Although direct, indirect, and circumstantial evidence of a role for autoimmunity in stable COPD patients has been identified, no cause-and-effect relationship between autoimmunity and the mechanisms of COPD has been firmly established in man. As such the potential contribution of an autoimmune response to the pathogenesis of COPD exacerbation is still being investigated and represents an area of active research**.** Many drugs targeting autoimmune responses are already available and the results of controlled clinical trials are awaited with great interest. The potential for measuring specific serum autoantibodies as biomarkers to predict clinical phenotypes or progression of stable COPD is promising.

Immune system in the normal adult human lung

Normal adult human lungs contain ~$30x10^9$ lymphocytes representing 7% of the total number of lymphocytes of the body. The lungs represents, therefore, a major lymphoid organ with a complex

functional organization that is only partially understood.[1,2]

There is a prevalence of $CD3^+$ T-cells, that, together with the gut, comprises ~14-15% of the total number of $CD4^+$ and $CD8^+$ cells of the body.[2,3] The $CD4^+/CD8^+$ lung ratio is ~1, with a high prevalence of $CD103^+$/integrin$\beta7^+/CCR5^+$ memory T-cells but with only a minority of $CD3^+TCR\gamma\delta^+$ ($\gamma\delta$T) cells.[3,4] The latter can influence B cell differentiation, control levels of circulating immunoglobulins and affect autoantibody production.[5] Both $CD27^+/CCR5^+$ and $CD27^-$ $CCR5^+$ resident memory cells (Trms) are found primarily in the lung whilst $CD69^+CD103^-$ effector memory (Tem) cells are also abundant in the lung.[4] T-helper (Th)-cells cannot be easily separated into distinct lineages in human tissues, as often secrete multiple Th-cell-associated cytokines. However, Th1 [interferon (IFN)-γ^+]-cells are found at the highest frequencies in the normal human lung.[4] It is noteworthy that only a small fraction (<6%) of these Th-cells are interleukin(IL)-4^+ (Th2), IL-9^+ (Th9), IL-10^+ (Th10), IL-$17A^+$ (Th17) or IL-22 (Th22) whereas around 50% of the Th-cells are granulocyte-macrophage colony-stimulating factor $(GM\text{-}CSF)^+$ that may protect against autoimmune diseases.[4,6] Th17 cells may play a pathogenic role in autoimmune diseases through the actions of IL-17A, IL-21 and IL-22 whereas $CD4^+/CD25^+$ forkhead box P3 $(FOXP3)^+$ T-regulatory (Treg) cells are critical for sustaining immune tolerance through IL-10 and transforming growth factor-β production.[7]

CD19+ B-cells ($CCR6^+CCR7^+CXCR5^+$) represent less of 5% of all lymphocytes whereas natural killer (NK, $CD3^-/CD56^+$) and NKT ($CD3^+/CD56^+$) cells contribute ~25% of all lymphocytes.[3,4]

Constitutive bronchus-associated lymphoid tissue (BALT) is not usually present in healthy human adults whereas inducible BALT (iBALT), an ectopic tertiary lymphoid structure composed of highly organized T- and B-cell zones with proliferating lymphocytes that forms in the lung in response to inflammation, including autoimmunity, can be found throughout the human lung. Both BALT and iBALT acquire antigens from the airways and initiate local immune responses maintaining memory cells in the lung.[8]

Autoimmune responses in the pathogenesis of stable COPD

Chronic obstructive pulmonary disease (COPD), a leading cause of morbidity and mortality worldwide, is caused by complex interactions between environmental factors (particularly cigarette smoking) and genetic factors. Long term cigarette smoking causes >90% of COPD in Westernized countries. However, only a fraction of chronic heavy smokers develop symptomatic COPD by the age of 80 years.[9]

The progressive chronic airflow limitation in COPD is due to two major pathological processes: remodelling and narrowing of small airways and destruction of the lung parenchyma with consequent loss of the alveolar attachments of these airways due to pulmonary emphysema. This results in diminished lung recoil, higher resistance to flow, and closure of small airways at higher lung volumes during expiration, with consequent air trapping in the lung. This leads to the characteristic hyperinflation of the lungs, which gives rise to the sensation of dyspnoea and decrease exercise tolerance. Both the small-airway remodelling and narrowing and the pulmonary emphysema are likely to be the results of chronic lung inflammation.[10]

COPD is characterized by an abnormal immune response in the lower airways with disease progression associated with infiltration of innate and adaptive inflammatory immune cells that form lymphoid follicles (LF). For this reason the inflammatory response present in the lower airways of stable COPD is considered an amplification of the inflammatory response to smoking that is seen in smokers with normal lung function.[9]

LF are rarely observed in the small airways of non-smokers, but they are present in the small airways of approximately 5% of smokers with normal lung function, as well as in smokers with mild to moderate COPD. Their prevalence increases sharply to 25–30% in severe and very severe COPD for unclear reasons. The LF in the small airways of COPD patients are large aggregates of B-cells, with interspersed $CD21^+$ and $CD35^+$ follicular dendritic cells surrounded by lower numbers of $CD4^+$ (80–90%) and few $CD8^+$ T-cells.[9,10]

The major T and B cell subsets involved in the pathogenesis of stable COPD (cell type and related functions) are summarized in table 1. [9,10]

There is growing evidence that autoimmunity has a role in the pathogenesis of stable COPD. Although direct, indirect, and circumstantial evidence of a role for autoimmunity in stable COPD has been identified (Fig 1), no cause-and-effect relationship between autoimmunity and COPD mechanisms has been established [11] and represents an area of intense active research.

We have not discussed any data on COPD exacerbations because of the absence of studies showing a role of autoimmunity in the pathogenesis of COPD exacerbations.

B cells and autoantibody-mediated lung damage in the pathogenesis of stable COPD

More of half century after the first demonstration of the presence of serum antibodies reactive against human lung tissues in patients with "idiopathic obstructive emphysema"[12] the role of B-cells and autoantibodies in the pathogenesis of stable COPD is still unknown. Although pulmonary emphysema usually only appears with increasing COPD severity, it can also occur in patients without airflow obstruction.[9,10] It is interesting to note that most of the evidence supporting the presence of an autoantibody-mediated autoimmune response in stable COPD patients has been gathered from studies of the more advanced grades of the diseases that are often associated to the presence of significant pulmonary emphysema. This suggests that autoantibodies may contribute to the pathogenesis of pulmonary emphysema.

B-cells localized in the peripheral lung of stable COPD are mainly immunoglobulin (Ig)M-bearing and IgD-negative, which suggests a degree of activation. Moreover, a predominant part of these cells is $CD27^{+}$, a marker for memory B-cells. In one study, where LF were isolated from the small airways of stable COPD patients, oligoclonal B-cells were find suggesting that they play a role in local antigen specific autoimmune responses.[9] B-cells organized in iBALT structures and macrophages accumulate in the lungs and contribute to cigarette smoke (CS)-induced pulmonary emphysema, and B cell-deficient mice is significantly protected against CS-induced emphysema.[13]

Natural (because they are produced at birth in the absence of exposure to foreign antigens) IgM autoantibodies (IgM-NAA) are present in the serum of the normal subjects and show poly-reactivity with low binding affinity, but with high avidity. This response provides time for the adaptive immune system to mount a highly specific immune response to foreign antigens lessening the necessity to produce highly specific IgG auto-antibodies, with the potential of causing autoimmune disease owing to their high-affinity binding.[14]

However, serum and/or lung IgM, IgG and less frequently IgA autoantibodies are often (but not always) found in both smokers with normal lung function and stable COPD patients. These are not disease-specific being directed against rheumatoid factor (RF), nuclei (ANA), lung, lower airways epithelium (including their cytokeratin 18 and 19), endothelium, anti-cyclic citrullinated peptide (anti-CCP) and/or extracellular matrix components (such as elastin, collagen I, and V, decorin) and many other epitopes (for example mutated citrullinated vimentin or CD80). In most cases their presence significantly correlates with smoking rather than with the degree of airflow limitation.[9,15-20 and references cited therein]

Carbonyl-modified proteins, arising due to oxidative/nitrosative stress (Fig 2), promote serum auto-antibody production in stable COPD.[21] The serum antibody titer against carbonyl-modified self-proteins significantly increases in patients with severe stable COPD compared with control subjects. Auto-antibody levels correlate inversely with disease severity and are predominantly IgG_1 in type. Deposition of activated complement in the vessels of peripheral COPD lung as well as autoantibodies against endothelial cells is also seen. Ozone-exposed mice, a model of COPD, show increased antibody titers to carbonyl-modified protein, as well as activated antigen-presenting cells in lung tissue and splenocytes sensitized to carbonyl-modified protein.[21] This confirmed previous observations showing the presence of serum and lung immune complexes in stable COPD patients.[17,22]

B-cell activating factor [BAFF, also termed B-lymphocyte stimulator (BLyS)] belongs to the tumor necrosis factor (TNF) family and is responsible for B-cell survival and maturation and its

overexpression is associated with autoimmune diseases.[23] BAFF is overexpressed in bronchiolar LF and in the small airways from stable COPD patients compared to control non-smokers but not in control smokers with normal lung function. BAFF overexpression creates a self-perpetuating loop contributing to COPD progression by promoting pulmonary B-cell survival and LF expansion.[24] Antagonizing BAFF in cigarette-smoke (CS)-exposed mice attenuates pulmonary inflammation and alveolar destruction.[25]

IL-6 (originally identified as a B-cell differentiation factor) is a multifunctional cytokine that may contribute to the pathogenesis of the autoimmune response observed in the lungs of severe stable COPD patients.[9,26]

IL-17A in the peripheral lung of patients with advanced COPD may contribute to disease progression and the development of lymphoid follicles via activation of CXCL12.[27] A major source of IL-17A is the $CD4^+$ T_H17 cell, which also releases IL-21 and IL-22, expresses the transcription factor retinoic acid–related orphan receptor γt (RORγt and called RORC2 in humans). Although Th17 cells are typically considered to be the principal source of IL-17, $CD8^+$ cells have also been shown to make this cytokine, and are termed 'Tc17'. In addition, several innate-acting lymphocytes such as IL-17^+ type 3 innate lymphoid cells (ILC3s) which lack an antigen receptor, γ_δT cells, some NKT cells, and TCRβ^+natural Th17 cells produce IL-17A. Collectively, these IL-17-producing cells are often termed type 17.[28]

In animal models microbiota may promotes chronic pulmonary inflammation by enhancing IL-17A expression and the subsequent synthesis of autoantibodies and an imbalance of the microbiome has been recently reported in bronchial biopsies of stable COPD patients. [29,30]

Cell-mediated autoimmune lung damage in the pathogenesis of stable COPD

The first evidence to the hypothesis that stable COPD may be caused by T-cell inflammation and autoimmunity derived from the presence of an increased number of activated signal transducer and activator of transcription (STAT)-4 and IFN-γ CD4+ T-cells in bronchial biopsies and BAL that correlate with decreasing lung function.[31]

This indicates that activated T-cells, through Th1 cytokines, along with CD8+ T-cells and innate immune cells recruited by Th1 cytokines, are damaging the lung.[32] Subsequently, severe emphysema has been associated with lung oligoclonal CD4+ and CD8+ T cells[33,34] and the presence of ADCC against bronchial epithelium in stable COPD.[17] In addition, in animal models chronic CS exposure generates pathogenic T-cells inducing a COPD-like disease.[35]

Cytotoxic pro-inflammatory $CD8^+$ T-cells and $CD8^+$ NKT-like cells are increased in the peripheral blood and airways of COPD patients, but only in the lungs of smokers with normal lung function. In COPD, these senescent cells express increased levels of the cytotoxic mediators, perforin and granzyme B, and the pro-inflammatory cytokines IFN-γ and TNF-α and have increased cytotoxicity toward lung epithelial cells.[36]

Production of these pro-inflammatory mediators by lung-resident $CD8^+$ T-cells may contribute to the pathogenesis of stable COPD.[9,26] In animal models of emphysema there is persistence of lung oligoclonal $CD8^+$ T-cells upon smoking cessation.[37] This may lead to chronic activation of macrophage subtypes that preclude proper healing of the lung and, thereby, contributing to pulmonary emphysema.[38]

Diagnostic implications

Autoantibodies may play a pathogenic role in the pathogenesis of COPD progression and in the pathogenesis of autoimmune emphysema (Fig 3). Furthermore, serum autoantibodies may act as biomarkers to predict clinical phenotypes or progression of stable COPD. A United States of America patent application (20160097778) has been filled on a method for determining COPD progression (and thus prognosis) and/or in selecting a personalised treatment, based on measuring serum autoantibody response to carbonylated vimentin (an intracellular intermediate filament expressed in mesenchymal cells) (http://www.freepatentsonline.com/y2016/0097778.html).

Therapeutic implications

In contrast to asthma, glucocorticoid treatment of stable COPD is rather ineffective in reducing airway inflammation and lung function decline.[39] However, long term withdrawal of inhaled glucocorticoids in stable COPD patients results in a significant rise in the number of $CD3^+$, $CD4^+$ and $CD8^+$ T-cells in the bronchial mucosa.[40] Inhaled glucocorticoids may reduce the adaptive immune response in stable COPD and may be more effective in patients with an increased B-cell response indicated by high autoantibody titer.[41] Unfortunately, this may also account for the small increased risk of pneumonias observed in stable COPD patients on high dose inhaled glucocorticoids.[26,39]

Macrolide antibiotics, such as azithromycin and erythromycin, have immunomodulatory/anti-inflammatory effects.[42] Long term treatment with these drugs reduce the frequency of exacerbations in patients with moderate to very severe COPD and a history of frequent exacerbations.[43] However, this is associated with an increased risk of adverse events and emergence of macrolide-resistance. Therefore, non-antibiotic macrolides with immunomodulatory/anti-inflammatory effects are under preclinical development.[42,44]

In addition there are many potential anti–B-cell–directed therapies that are in clinical use for the treatment of autoimmune diseases which have potential for the treatment of stable COPD patients. B-cell depletion by anti-CD20 monoclonal antibodies (mAbs) rituximab, ocrelizumab and ofatumumab have efficacy in multiple sclerosis.[45] CD20 regulates cell cycle progression in B-cells and in mice loss of CD20 functions leads to a 20-30% reduction in B-cell numbers with low serum IgG and normal IgM levels.[46] Epratuzumab is a B-cell-directed non-depleting anti-CD22 mAb, which results in reduced B-cell receptor (BCR) complex signalling by intensifying the normal inhibitory role of CD22 on the BCR and diminishing B-cell activation, that is in phase 3 clinical trials in patients with systemic lupus erythematosus (SLE).[47] BAFF plays a central role in the induction and maintenance of CS-induced pulmonary autoantibodies suggesting a therapeutic potential for BAFF blockade in limiting autoimmune processes associated with smoking.[48] Belimumab, a mAb anti-BAFF (reducing B-cell differentiation and survival) is the first drug to be

approved specifically for the treatment of SLE in more than 50 years.[49]

Anti-IL-6 blocking mAbs (including siltuximab and tocilizumab), although used in clinical trials of rheumatoid arthritis and cancers have not been tested on COPD patients.[26,39]

Secukinumab and ixekizumab are mAbs that selectively binds and neutralizes IL-17A blocking its binding to the IL-17 receptor (IL-17R) and have been approved for the treatment of psoriasis.[50] However, CNTO 6785, another anti-IL-17A mAb, was ineffective in a phase 2 randomised controlled trial (RCT) of stable COPD.[51] Brodalumab, a mAb blocking IL-17Rα, has been tested in patients with severe asthma. [26,39]

IL-23, a key regulator of Th17 cells, can be inhibited by specific mAbs (guselkumab and tildrakizumab) or ustekinumab, which blocks the shared p40 component of IL-12 and IL-23. These are all effective in the treatment of psoriasis.[26,39] Low molecular weight, cell-permeable RORC2 antagonists and novel RORC2-independent modulators of IL-17A have been identified.[52]

In addition, statin treatment reverses the IL-17A/IL-10 imbalance in the airways of stable COPD patients but, unfortunately, statins are clinically ineffective in the long-term treatment of these patients.[26]

Anti-CD3 (expressed on ~90% of lung T-cells) mAbs effectively treat autoimmune disease in animal models and show promise in clinical trials. The development of tolerance by anti-CD3 mAbs is related to the induction of Tregs.[53]

It is unlikely that mouse models of COPD will be more predictive than those reported to date. This demands the testing of novel therapies in RCTs in patients with stable COPD to demonstrate efficacy on COPD exacerbations and disease progression. Close monitoring of infectious and non-infectious adverse events is mandatory. In fact, blocking immune responses might be associated with reduced immunity to infections.

Conclusions

There is growing evidence that ultimately autoimmunity, like so many other facets of

pathophysiology linked with COPD, is likely to play a more central role in the progression of the disease in certain subgroups of COPD patients rather than a unifying central across all COPD. Both antibody and cell-mediated responses appear to be involved in autoimmune responses and in the development of lung damage. In particular, carbonyl-modified proteins may drive autoimmune mechanisms in COPD causing the characteristic small airways abnormalities and pulmonary emphysema. Drugs that target this autoimmune response are available and the results of controlled clinical trials are awaited with great interest. The potential for measuring specific serum autoantibodies as biomarkers to predict clinical phenotypes, progression of stable COPD or drug response is promising.

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Table 1. Major T and B cell subsets involved in the pathogenesis of stable COPD.

Cell Type	**Function**	**Expression**	**Comments**
B cell	Humoral immunity	□	Identified in the secondary lymphoid follicles of the COPD lung as mainly IgM+, IgD-
$CD57^+$ B cel	Memory B cells	□	Observed mainly in the lower airways GOLD 3 and 4 grades
$CD3^+$ T cells	Cell mediated immunity	□	Includes both $CD4^+$ and $CD8^+$ T cells. Numbers in the lower airways are significantly elevated in GOLD 3 and 4 compared to GOLD 1 and 2 grades.
$CD8^+$ T cell	Cytotoxic cellular immunity	□□	Elevated in blood and lower airways. These cells are highly activated expressing perforin and granzyme B.
$CD4^+$ T cell	Provide help adaptive immune response	□	Increased mainly in blood.
$CD8^+$ - IFNγ/Il-4 ratio	Defines Th1 (more IFNγ) versus Th2 (more IL-4) immune response	□	Their ratio in the lower airways compartments can define whether the immune response is directed towards intracellular pathogens (Th1) as opposed to extracellular

			pathogens (Th2).
CD8/CD4 ratio	Indicative of abnormal immune response	□	Most significant increase in the lower airways of COPD in GOLD 3 and 4 grades where ratio = 4 (a normal ratio = 0.5). This increased CD8/CD4 ratio is associated with the severity of airflow limitation in COPD.
Th17 cells	B cell maturation, also facilitates neutrophils and macrophage recruitment for mucosal immunity	□□	High levels of IL-17 are strongly linked to development of autoimmune disorders. In the lower airways of stable COPD, only 5% of IL-17 is from Th17 cells, the other 95% of IL-17 is from $CD31^+$ endothelial cells.
$CD25^+$ T_{reg} cells	Maintains the immunological tolerance by suppressing activated self-reactive T cells	□□	Decreased in the lower airways of COPD. All the T_{reg} cell express the master transcription factor regulator FoxP3, its expression in the lower airways is inversely correlated to airflow limitation.

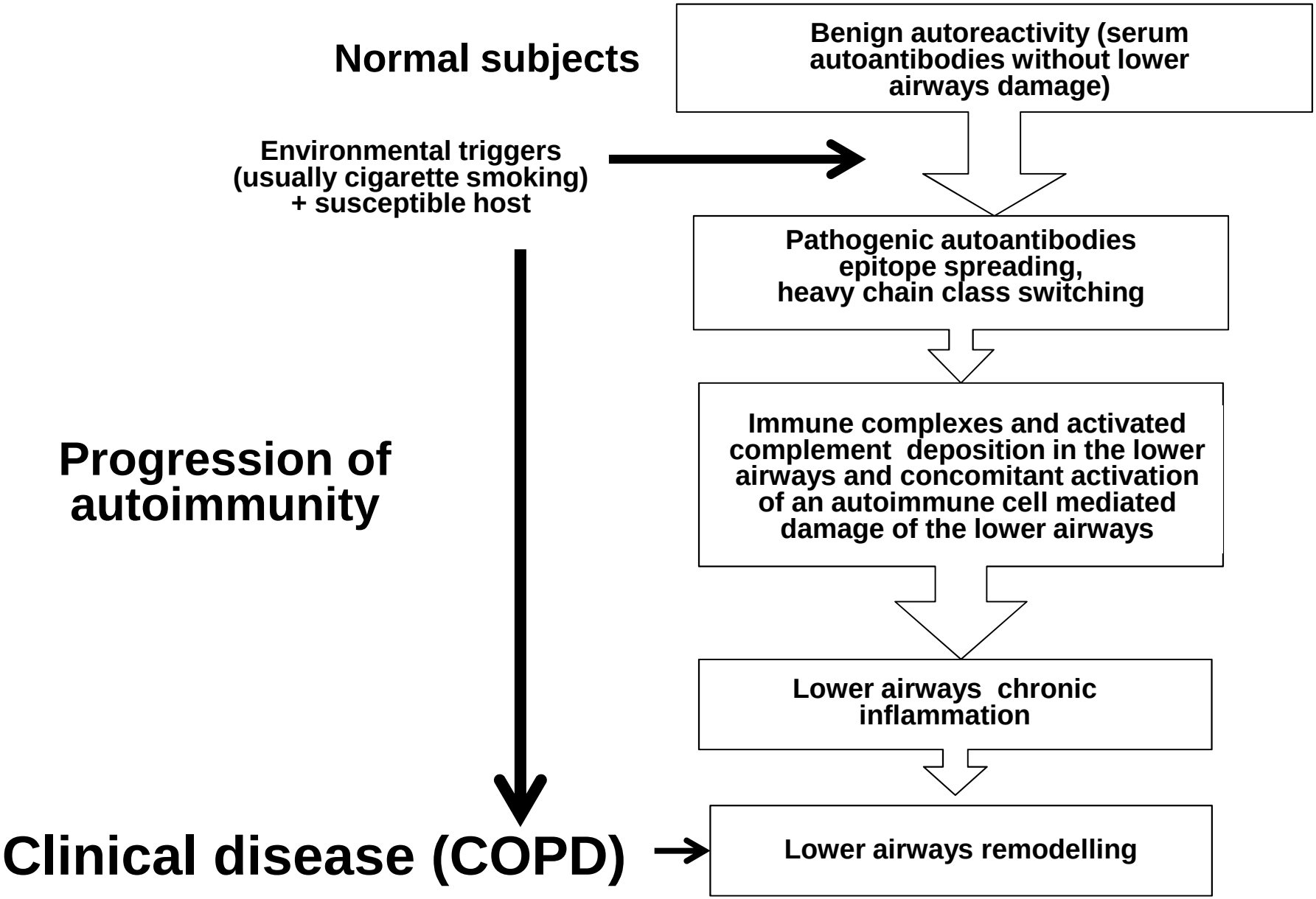
Obtained with the data from references 9, 10.

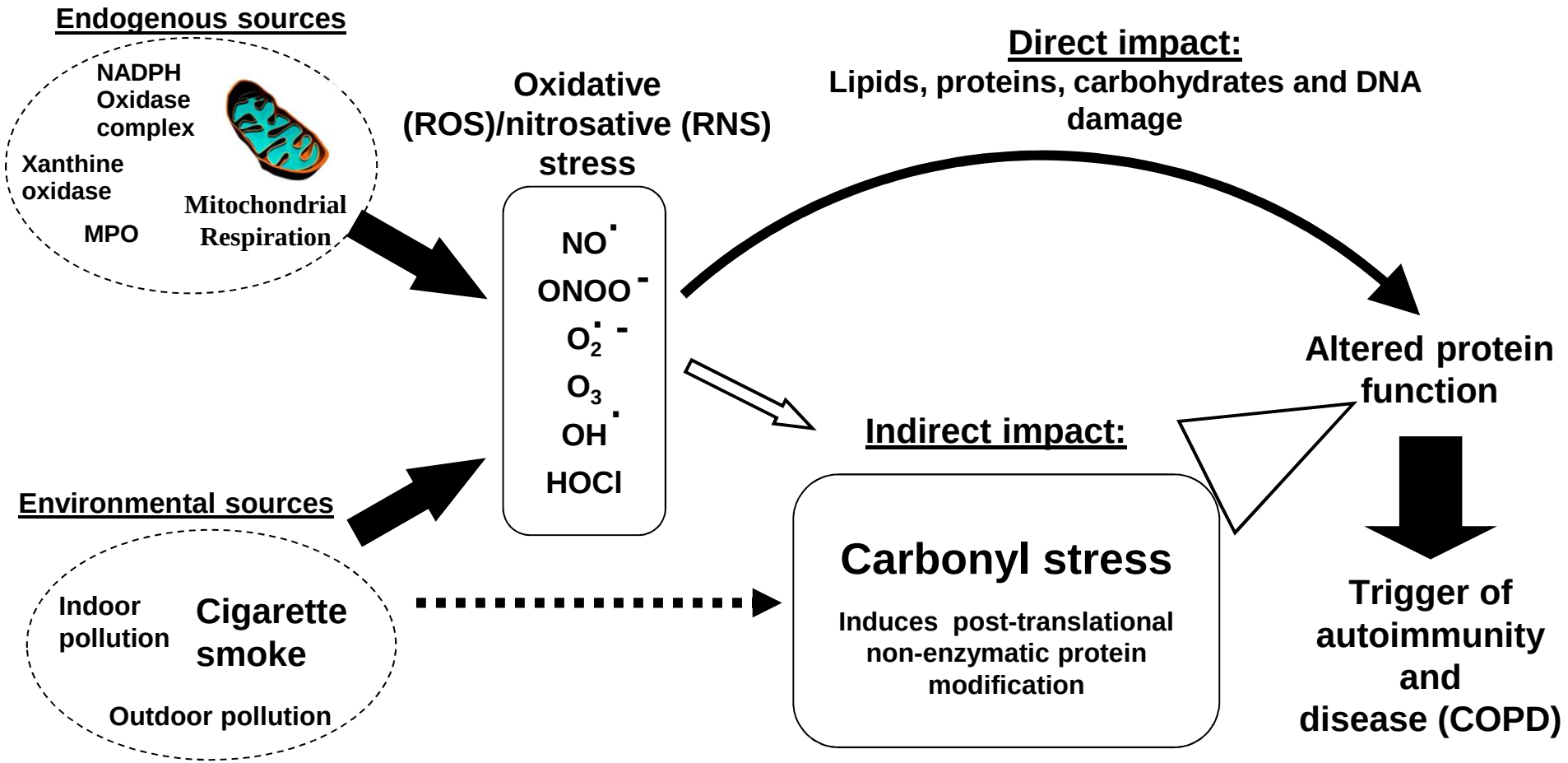
Figure legends

Figure 1. Autoimmunity develops in chronic obstructive pulmonary disease (COPD) when environmental triggers (usually cigarette smoking) transform the production of benign autoantibodies to pathogenic antibodies in susceptible subjects. With the progression of the autoimmune response there is the deposition of immune complexes within the lower airways and the local activation of cell mediated autoimmune damage which causes lower airway chronic inflammation. This is followed by a slowly progressive remodelling of the lower airways (chronic bronchiolitis and pulmonary emphysema) and by the clinical diagnosis of the disease (COPD) (slightly adapted from reference 9 under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)

Figure 2. Oxidative (ROS) and nitrosative (RNS) stress from environmental (for example cigarette smoking, air pollution, indoor biomass smoke) and endogenous sources [for example myeloperoxidase (MPO), mitochondrial respiration, nicotinamide adenine dinucleotide phosphate reduced oxidase complex, xanthine oxidase] may causes direct tissue damage through lipids, proteins, carbohydrates and deoxyribonucleic acid (DNA) damage. However may also causes the formation of carbonyl stress that through post-translational, non-enzymatic, modifications on proteins can alter their function and result in the formation neo-autoantigens triggering an autoimmune response in the lower airways of smokers that in susceptible subjects is associated with a slowly progressive damage of the lower airways and development of chronic obstructive pulmonary disease (COPD). ROS =reactive oxygen species. RNS=reactive nitrogen species.

Figure 3. Diagnostic and therapeutic implications of the pathogenic role of autoimmunity in stable COPD patients.




Endogenous sources
NADPH Oxidase complex
Xanthine oxidase
MPO
Mitochondrial Respiration
Oxidative (ROS)/nitrosative (RNS) stress
NO·
ONOO-
O2·-
O3
OH·
HOCl
Direct impact:
Lipids, proteins, carbohydrates and DNA damage
Altered protein function
Indirect impact:
Environmental sources
Indoor pollution
Cigarette smoke
Outdoor pollution
Carbonyl stress
Induces post-translational non-enzymatic protein modification
Trigger of autoimmunity and disease (COPD)